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Medicine, University of

CULTURED ENDOTHELIAL CELLS DERIVED FROM THE HUMAN ILIAC ARTERIES

The purpose of this cell culture study was to establish *in vitro* cell cultures derived from the endothelium of human great arteries from individuals with varying degrees of atherosclerotic disease. To do this, cells derived from the endothelium of human iliac arteries were cultured *in vivo*. The cells were isolated, grown and subcultured in HEPES buffered Medium 199 supplemented with 20% heat inactivated human whole blood serum, human alpha-thrombin, and commercial endothelial cell growth supplement derived from bovine brain. The cells were viable in culture for 8 to 10 passages at a split ratio of 1:3. After the 10th passage, the cells began to enlarge and their growth rate was reduced. No cultures were viable after the 12th passage. The cells were determined to be of endothelial origin by their morphology at confluence, their ultrastructural characteristics, the production and release of factor VIII-related antigen, and by their maintenance of a surface that prevented platelet attachment. The cultured arterial endothelial cells released prostacyclin in response to challenge with thrombin and protamine sulfate, but not in response to bradykinin or the platelet-derived growth factor. Although the cultures described here were derived from patients with varying degrees of atherosclerotic disease, there were no significant differences in morphological or physiological parameters among the cultures or in comparison with commonly studied cells derived from human umbilical veins.

Glassberg, M. K., Bern, M. M., Coughlin, S. R., Haudenschild, C. C., Hoyer, L. W., *Antoniades, H. N.*, and Zetter, B. R.

In Vitro 18(10):859-866, 1982.

Other support: National Institutes of Health.

From the Department of Physiology and Surgery, Children's Hospital Medical Center, Harvard Medical School, Boston; Department of Medicine, New England Deaconess Hospital, Harvard Medical School, Boston; Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge; Mallory Institute of Pathology, Boston University School of Medicine, Boston; Department of Medicine, University of Connecticut Health Center, Farmington; Center for Blood Research and Harvard School of Public Health, Boston.

INCORPORATION OF 7-KETOCHOLESTEROL BY PLASMA MEMBRANES FROM PORCINE AORTIC ENDOTHELIAL CELLS

In this paper evidence is presented that 7-ketocholesterol (7KC) is incorporated into the plasma membranes of endothelial cells in excised porcine aortas in molar amounts comparable to the native cholesterol present in the membrane. These experimental findings indicate that simple adsorption of 7KC does not play a role in the results obtained. Instead 7KC appears to be inserted into the lipid bilayer of the membranes. This is largely independent of membrane cholesterol content and is not due to substitution of the native membrane cholesterol. A significant increase in the total sterol-to-phospholipid ratio is obtained with 7KC-exposure without significant changes in the cholesterol-to-phospholipid ratio. In summary, therefore, these data suggest that the net incorporation of substantial amounts of 7KC in the plasma membranes is very likely due to a process of insertion of the 7KC molecules into the lipid bilayer matrix of the plasma membrane.

Santillan, G. G., Zak, I. and *Bing, R. J.*

Artery 11(2):119-135, 1982.

Other support: The Hoover Foundation.

From the Huntington Memorial Hospital, Huntington Medical Research Institutes, and the California Institute of Technology, Pasadena.

MICROCIRCULATION OF LEFT ATRIAL MUSCLE, CEREBRAL CORTEX AND MESENTERY OF THE CAT: A COMPARATIVE ANALYSIS

Capillary morphometry and topography and O_2 supply to the tissue are intimately related to the capillary functions of maintaining tissue oxygenation and responding rapidly to changing oxygen demands. To investigate these associations, comparative analyses were carried out by means of transillumination on the geometry, topography and morphometry of microcirculation in the cerebral cortex, left atrial muscle and mesentery of the cat using computer analysis. In addition, specific types of capillary distribution (concurrent, countercurrent and asymmetric distribution) in these three organs were ascertained from images visualized on films. These parameters were related to their role in tissue oxygen supply. It was found that mean capillary diameter, mean intercapillary distance, total capillary length, and total capillary surface area differed significantly among the three organs. Differences in mean capillary tortuosity between cerebral cortex and left atrial muscle and between left atrial muscle and mesentery also were significant. Mean capillary tortuosity in mesentery and cerebral cortex was of equal magnitude. In the cerebral cortex a high degree of tortuosity and asymmetric capillary distribution favor tissue oxygenation. A similar situation exists in left atrial muscle. In the mesentery, the combination of high capillary tortuosity and concurrent capillary arrangement is unfavorable for tissue oxygenation.

Chang, B-L., Yamakawa, T., Nuccio, J., Pace, R., and *Bing, R. J.*

Circulation Research 50(2):240-249, 1982.

Other support: The Hoover Foundation.

From the Huntington Memorial Hospital, Huntington Institute of Applied Medical Research, and the California Institute of Technology, Pasadena.

EFFECT OF ALCOHOL ON THE HEART AND CARDIAC METABOLISM

This report was compiled to delineate the disturbances in biochemistry of heart muscle when exposed to ethanol. The disturbances are many. All elements of cellular substructure are effected. In plasma membranes, $(Na^+ + K^+)$-activated ATPase (EC 3.6.1.3) is inhibited. Mitochondrial damage consists of diminished respiratory function and calcium uptake and binding. High-energy phosphates remain intact. Alcohol also effects the malate-aspartate shuttle. Acetaldehyde, a metabolite of ethanol, has a direct effect on myocardial protein synthesis through microsomal inhibition; however, the development of cardiac hypertrophy is not affected. Malfunction of sarcoplasmic reticulum is evidenced by disturbances in calcium binding and uptake. Effects of ethanol on the contractile machinery are deficiencies in the turnover rate of chemical into mechanical energy (diminished V_{max}), and in the number of cross-bridges formed (P_o). It increases stiffness of series elastic elements. There is diminished fatty acid

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Bing, R. J.

Federation Proceedings 41:2

Other support: U. S. Public

From Huntington Memorial
Research, Pasadena, CA.

INHIBITORY EFFECT OF
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Journal of Molecular and Cel

Other support: The Hoover F

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MICROCIRCULATION IN

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oxidation with increased esterification. The involvement of CoA synthetase (EC 6.2.1.1), palmityl-carnine transferase (EC 2.3.1.7), and pyruvate dehydrogenase complex in disturbed fatty acid oxidation is not certain. The role of catalase in myocardial ethanol oxidation was also examined. Ethanol activates myocardial catalase-H_2O_2 complex (EC 1.11.1.6). The biochemical basis of fetal alcohol syndrome is low hepatic alcohol dehydrogenase (EC 1.1.1.1) activity during fetal life.

Bing, R. J.

Federation Proceedings 41:2443-2446, 1982.

Other support: U. S. Public Health Service and The Hoover Foundation.

From Huntington Memorial Hospital and Huntington Institute of Applied Medical Research, Pasadena, CA.

INHIBITORY EFFECT OF A CALCIUM ANTAGONIST (DILTIAZEM) ON AORTIC AND CORONARY CONTRACTIONS IN RABBITS

The *in vitro* experiments reported here were carried out on 18 coronary arterial and 50 aortic strips from 26 New Zealand white rabbits in an attempt to investigate the effect of a slow calcium channel blocker, diltiazem, on the isometric contractions induced by certain prostaglandins, $PGF_{2\alpha}$ and PGE_2, 5-hydroxytryptamine (5-HT) and nicotine on the isolated strips. Results showed that diltiazem inhibited contraction induced by 5-HT and by the two prostaglandins $PGF_{2\alpha}$ and PGE_2 in coronary arteries only. Specifically these experiments demonstrate that a slow calcium channel blocker, diltiazem, effectively inhibits contractions of coronary artery strips induced by $PGF_{2\alpha}$, PGE_2 and 5-HT and also effectively diminishes the isometric contractions induced by nicotine in aortic strips. The contractions induced by nicotine, which was effective in aortic smooth muscle only, are also markedly inhibited by diltiazem as well as by prozosin an α_1 adrenergic inhibitor. Rauwolscine, an α_2 inhibitor, has little effect.

Sato, M., Ohashi, M., Metz, M. Z., and *Bing, R. J.*

Journal of Molecular and Cellular Cardiology 14(12):741-744, 1982.

Other support: The Hoover Foundation.

From Huntington Medical Research Institutes and Huntington Memorial Hospital, Pasadena, CA.

MICROCIRCULATION IN MYOCARDIUM AND CEREBRAL CORTEX

The cerebral microcirculation has been studied by a variety of methods. While most of the early findings were based on histological observations, recently more pertinent information of cerebral microcirculatory flow has been noted by direct observation. However, most of these determinations have been made on the pial vessels by epiillumination. In contrast, few *in vivo* observations of the intracortical cerebral microvasculature have been reported. The report presented here is concerned with findings based on a technique which permitted visualization of the cortical microcirculation in the live anesthetized cat. The following subjects were studied: I. Capillary distribution, II. Comparative analysis of microcirculation in heart and brain, and III. The effect of changes in systemic blood pressure induced by hemorrhage on microcirculatory autoregulation in the cerebral cortex. Many references are considered here and much calculation is presented and carried out. For calculation of capillary

morphometry and geometry, a computer program was designed to run on PDP 11-03 minicomputers. Results of several studies show that there may be a significant interaction between certain types of capillary curving and characteristics of formed blood elements. It is conceivable that more rigid cells may resist any further deformation in narrow capillary curves. The resulting pressure differential affects not only red cell velocity within the same capillary segment, but causes instantaneous redistribution of pressure gradients in adjoining capillaries. An important observation from the comparative analysis of microcirculation in the myocardium, cerebral cortex and mesentery is that capillary morphometry and topography and O_2 supply to the tissue are intimately related.

Bing, R. J. and Chang, B-L.

In: Dintenfass, L., Julian, D. G. and Seaman, G. V. F. (eds.): *Heart Perfusion, Energetics, and Ischemia*, New York: Plenum Publishing Corporation, 1983, pp. 157-178.

Other support: The Hoover Foundation.

From the Department of Experimental Cardiology, Huntington Medical Research Institutes, Huntington Memorial Hospital, Pasadena, CA.

THE ACTION OF DILTIAZEM ON VASCULAR SMOOTH MUSCLE AND ON PROTECTION OF THE ISCHEMIC (DOG) AND ISCHEMIC REPERFUSED (RAT) HEART

This article, which was dedicated to Dr. Albert Fleckenstein on the occasion of his 65th birthday, is devoted primarily to the effects of diltiazem, a calcium antagonist, on vascular smooth muscle of rabbits, and to the drug's effect in protecting the ischemic and ischemic reperfused myocardium. This article is very apropos since Fleckenstein in 1969 was the first to point out that Isoptin® prevents myocardial necrosis induced by isoproterenol. He mentioned for the first time the possible role of Ca^{++} antagonism: "the future will show whether or not there is a rational way to prevent the mechanism of necrosis formation in myocardium by means of calcium antagonists." The results presented here bear out this prediction. In summarizing the material in this paper, the prediction of Fleckenstein in 1968 that the vascular smooth cell is highly susceptible to the action of Ca^{++} antagonists has been proved by the antagonistic effect of Diltiazem on the prostaglandins $PGF_{2\alpha}$ and PGE_2. These findings of the protection of the ischemic and ischemic reperfused myocardium also are in line with the original idea of Fleckenstein, first proposed in 1968: Ca^{++} antagonists prevent myocardial damage by protecting the heart muscle from excessive activation of Ca-dependent intracellular ATP-ases and from Ca^{++} overload. This ingenious view expressed in the sixties, which for the first time stressed the significance of myocardial Ca^{++} overload, serves as a valid explanation of the protection of the ischemic and ischemic reperfused myocardium.

Bing, R. J. and Chang, B-L.

In: *Fleckenstein's 65th Birthday Celebration Volume*, New York: Gustav Fischer Verlag, 1983, pp. 5-13.

Other support: The Hoover Foundation.

From the Huntington Medical Research Institutes and Huntington Memorial Hospital, Pasadena, CA.

BIOLOGICAL SIGNIF

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Other support: National

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ALTERATION IN PLA
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BIOLOGICAL SIGNIFICANCE OF HEPARAN SULFATE PROTEOGLYCANS

Heparan sulfate chains have been found to be present as protein-bound carbohydrate chains both at the cell surface and in the supernatant growth medium of rabbit endothelial cultures, thus raising the possibility that these cell products may play a role in two of the most typical functions of the endothelium, the regulation of the exchanges between blood and tissues and the ability to maintain blood fluidity. In earlier experiments, several lines of evidence seem to imply that the heparan sulfate proteoglycans of these cells possess several distinct features that differentiate them from similar molecules synthesized by other cells of the vessel wall. Now, the overall studies considered in this paper suggest that an endothelial cell inhibitor (presumably one of the slower-moving heparan sulfate proteoglycans) interacts with one of the coagulation enzymes of the thromboplastin pathway and by so doing inhibits the conversion of prothrombin to thrombin. Through this mechanism, heparan sulfate proteoglycans may play an essential role in imparting the property of blood compatibility to the endothelial cell surface. The function of the other heparan sulfate species (the electrophoretically faster-moving) is at present unknown. It can be hypothesized that, given the multiplicity of structures that these cell products exhibit, they participate in a wide range of endothelial cell functional activities by either inhibiting or activating enzymes that are an important part of certain blood homeostatic mechanisms or by binding certain proteins of biological importance that influence the functions of the endothelium or its interaction with other blood components. Thus, the generic name of "proteolgycans" comprises a number of species whose structures and functions may be widely different.

Buonassisi, V. and Colburn, P.

Annals of the New York Academy of Sciences 401:76-84, 1982.

Other support: National Heart, Lung, and Blood Institute.

From the Department of Biology, University of California at San Diego, La Jolla.

ALTERATION IN PLASMA PROTEINS AND PLATELET FUNCTIONS WITH AGING AND CIGARETTE SMOKING IN HEALTHY MEN

A longitudinal clinical study was started several years ago to investigate the effect of normal aging and chronic smoking on blood coagulation and platelet function. The volunteer subjects used for this study were obtained through the computer research facility of the Normative Aging Study of the Veterans Administration Outpatient Clinic in Boston. As reported here, blood samples were obtained on four different occasions from 18 cigarette smoking and 34 nonsmoking healthy men (age 40-69) and analyzed to assess age-and smoking-associated changes in plasma proteins, blood coagulation and platelet functions. Results showed that collagen-induced platelet aggregation was significantly increased with aging in non-smokers. Significant changes in chronic smokers were increases in platelet count and fibrinogen in plasma; elevation of platelet factor-3 (PF-3) activity in platelet-poor plasma (PPP); increase in serum levels of α_1-antitrypsin, orosomucoid, haptoglobin and properdin factor B; and shortening of the lag period of collagen-induced platelet aggregation. Filtration of PPP through Millipore filters removed PF-3 membranes. The differences in PF-3 activities

in filtered plasma were no longer significant between smokers and nonsmokers. Results suggest that chronic smokers have higher levels of acute phase proteins reflecting underlying inflammatory processes, and higher levels of PF-3 activity in plasma due to liberation of PF-3 membranes from platelets.

Chao, F. C. et al.

Thrombosis and Haemostasis 47(3):259-264, 1982.

Other support: National Institutes of Health and the VA Medical Research Service.

From the Center for Blood Research, Departments of Medicine and Pediatrics, Harvard Medical School, and the Veterans Administration Outpatient Clinic, Boston.

EVIDENCE FOR CALCIUM-SENSITIVE STRUCTURE IN PLATELET THROMBOSPONDIN

The isolation of thrombospondin in the presence of calcium is reported in this paper, where it is also shown that thrombin and trypsin proteolysis, as well as the sedimentation coefficient, intrinsic viscosity, and electron microscopic appearance of thrombospondin, are sensitive to the presence of calcium. As shown here, when thrombospondin, which was purified in the presence of EDTA, was exposed to thrombin, high molecular mass fragments were observed at 175,000, 160,000, 145,000, and 135,000 daltons. In contrast, when the supernatant from thrombin-treated platelets was incubated with additional thrombin, only the 160,000-dalton fragment of thrombospondin was produced. The results of thrombin digestion of supernatant samples under varying ionic conditions suggested that the addition of EDTA during isolation of thrombospondin disrupted native calcium-sensitive structure in thrombospondin. To preserve these structures, thrombospondin was purified by heparin affinity chromatography. The peptide pattern produced by thrombin digestion of purified thrombospondin is different from that observed for thrombospondin in the supernatant from thrombin-treated platelets. Dialysis experiments indicate that the peptide pattern produced by thrombin is dependent upon the presence of calcium. Other results presented here are consistent with low angle rotary shadowing data, which indicate that thrombospondin appeared to be comprised of three to four well defined nodular domains connected by thinner flexible regions.

Lawler, J., *Chao, F. C.* and Cohen, C. M.

The Journal of Biological Chemistry 257(20):12257-12265, 1982.

Other support: National Institutes of Health.

From the Department of Research, St. Elizabeth's Hospital of Boston, Tufts University School of Medicine, Boston, and the Center for Blood Research, Boston.

ASSAY OF PREKALLIKREIN IN HUMAN PLASMA: COMPARISON OF AMIDOLYTIC, ESTEROLYTIC, COAGULATION, AND IMMUNOCHEMICAL ASSAYS

This methodological study compares three functional assays for prekallikrein using coagulation, esterolytic and amidolytic substrates with a radial immunodiffusion

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Fisher, C. A., Schmaier

Blood 59(5):963-970, 19

Other support: National

From the Thrombosis Re
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FACTOR XI ANTIGEN

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Research, Boston.

OMPARISON OF
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l assays for prekallikrein
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reference assay in three subject populations—normals, women receiving oral contraceptives and patients with severe liver disease. The amidolytic assay, which was designed to measure prekallikrein in plasma, uses the substrate H-D-Pro-Phe-Arg-p-nitroaniline-HCl. At a substrate concentration of 1mM, the amidolysis of purified kallikrein at 1 coagulant unit/ml was observed to be 2.47 μmole/min/ml. Conditions for plasma kallikrein activation were optimized to approach complete activation when compared to the amidolytic activity of the purified plasma kallikrein. Activated plasma prekallikrein had 78% of activity of purified kallikrein at plasma concentration. Comparison of this amidolytic assay with immunochemical, esterolytic, and coagulant assays of the three subject populations showed good correlation both in normals and in the patient groups between the amidolytic and esterolytic assays. Each enzymatic assay correlated with the immunochemical assay. However, comparison of each of these assays with the coagulant assay showed no significant correlation due to the large inherent error of the latter assay. This standardized plasma prekallikrein amidolytic assay should facilitate studies of plasma prekallikrein concentration in physiologic and pathologic conditions and help identify activation of the contact phase of coagulation in disease states.

Fisher, C. A., Schmaier, A. H., Addonizio, V. P., and *Colman, R. W.*

Blood 59(5):963-970, 1982.

Other support: National Institutes of Health.

From the Thrombosis Research Center and the Department of Medicine, Hematology/Oncology Section, Temple University Health Sciences Center, Philadelphia, and the Division of Cardiothoracic Surgery, Department of Surgery, University of Pennsylvania School of Medicine, Philadelphia.

FACTOR XI ANTIGEN AND ACTIVITY IN HUMAN PLATELETS

Previous studies have shown that factor XI activity resides in the plasma membrane fraction, is not inactivated by anti-factor-XI antibody and is present in platelets from a hemostatically normal patient with congenital absence of plasma factor XI. In the present attempt to further explore the nature of the intrinsic factor XI activity of platelets, washed platelets, contaminated with less than 0.20% plasma factor XI, were examined for the presence of factor XI antigen and activity. These platelets contained a factor XI-like coagulant activity that remained constant after successive washes. By means of indirect immunofluorescence, a monospecific antibody to factor XI showed specific staining of both normal platelets and platelets from patients deficient in plasma factor XI. Radiolabeled Triton extracts of washed platelets and labeled purified factor XI solutions were analyzed for factor XI antigen by Staph A immunoprecipitation analysis using antibody to purified plasma factor XI followed by SDS gel electrophoresis. On unreduced gels, the platelet material ran as a single band having an apparent molecular weight of 220,000 daltons, whereas purified plasma factor XI gave a single band at 160,000 daltons. On reduced gels, the platelet material analyzed as a single band at 52,000 daltons, whereas purified factor XI gave a single band of 80,000 daltons. These and other results support the view that platelet factor XI is functionally and antigenically similar to plasma factor XI but different in molecular weight and isoelectric point. In addition, the presence of factor XI activity and antigen in the

platelets of three hemostatically normal individuals with no detectable plasma factor XI activity or antigen supports the conclusion that the platelet material is not of plasma origin and that it may substitute for plasma factor XI in hemostasis.

Tuszynski, G. P., Bevacqua, S. J., Schmaier, A. H., *Colman, R. W.*, and Walsh, P. N.

Blood 59(6):1148-1156, 1982.

Other support: Department of Health, Education and Welfare.

From the Thrombosis Research Center, Temple University School of Medicine, Philadelphia.

EFFECT OF HEPARIN ON THE INACTIVATION RATE OF HUMAN FACTOR XIa BY ANTITHROMBIN-III

The contribution of heparin toward the inhibition of factor XIa by antithrombin III in purified systems and in plasma was assessed here using amidolytic and coagulant assays. At therapeutic heparin concentrations (1U/ml), no potentiating effect on this reaction was found, although inhibition of the amidolytic activity of thrombin by purified antithrombin-III was enhanced at least 20-fold by the same concentration of heparin. Furthermore, despite the ability of heparin (1U/ml) to increase the inactivation rate of thrombin by plasma, no acceleration of the rate of inhibition of factor XIa by plasma was observed. Similar results were found when the inhibition of factor XIa was monitored with a coagulant assay after first removing heparin. Only at heparin concentrations of 5 and 10 U/ml, was a 2-and 4-fold increase in the inactivation rate of factor XIa by purified antithrombin III observed. Therefore, in both purified systems as well as plasma, heparin, at concentrations observed in clinical practice, does not accelerate the inactivation rate of human factor XIa by antithrombin-III.

Scott, C. F., Schapira, M. and *Colman, R. W.*

Blood 60(4):940-947, 1982.

Other support: National Institutes of Health.

From the Thrombosis Research Center and Hematology/Oncology Section of the Department of Medicine, Temple University Health Sciences Center, Philadelphia.

PREKALLIKREIN ACTIVATION AND HIGH-MOLECULAR-WEIGHT KININOGEN CONSUMPTION IN HEREDITARY ANGIOEDEMA

Three unrelated patients with hereditary angioedema were studied in this attempt to determine whether activation of the contact phase of coagulation could be detected in peripheral blood during acute attacks of this disease. It has been known before that patients with hereditary angioedema lack $C\bar{1}$ inhibitor, a plasma α_2-glycoprotein that inhibits both the proteolytic action of $C\bar{1}$, the activated first component of the complement system, and the activity of components of the contact phase of coagulation: kallikrein, factor XI_a, and factor XII_a. Such patients have been shown to have low levels of C4 and C2, the natural substrates for $C\bar{1}$, but the levels were not correlated with the presence of symptoms. In the three-patient study presented here, it was found

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Schapira, M., Silver, L. D.,
J. G., and *Colman, R. W.*

The New England Journal o

Other support: Swiss Natior
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From the Thrombosis Rese
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PURIFIED HUMAN PLAS
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The effect of highly p
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PMN aggregation by plasma

Schapira, M., Despland, E.,

Journal of Clinical Investig

Other support: National Inst
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From the Division de Rhum
zerland; Temple University
of Pediatric Hematology-
Indianapolis.

that during a symptomatic period the patients had decreased levels of prekallikrein, a substrate for the activated forms of factor XII, and reductions in high-molecular-weight kininogen, a substrate for plasma kallikrein. These observations suggest that zymogens of the contact system are activated during attacks of hereditary angioedema and that some of the clinical manifestations may be mediated through products of this pathway, such as kinins.

Schapira, M., Silver, L. D., Scott, C. F., Schmaier, E. H., Prograis, L. J., Jr., Curd, J. G., and *Colman, R. W.*

The New England Journal of Medicine 308(18):1050-1054, 1983.

Other support: Swiss National Science Foundation, American Heart Association and the National Institutes of Health.

From the Thrombosis Research Center and the Department of Medicine, Temple University School of Medicine, Philadelphia, and Scripps Clinic and Research Foundation, La Jolla, CA.

PURIFIED HUMAN PLASMA KALLIKREIN AGGREGATES HUMAN BLOOD NEUTROPHILS

The effect of highly purified human plasma kallikrein on polymorphonuclear leukocytes (PMN) is described in this paper, using cell aggregation and oxygen consumption as criteria for neutrophil activation. Results of these studies show that exposure of human blood PMN to purified active plasma kallikrein resulted in PMN aggregation when kallikrein was present at concentrations ranging from 0.4 to 0.6 U/ml (0.18-0.27 μM). Kallikrein-induced PMN aggregation was not mediated through C5-derived peptides, because identical responses were observed whether or not kallikrein had been preincubated with an antibody to C5. Moreover, kallikrein was specific for aggregating PMN, because no aggregation was observed with Factor XII active fragments, Factor XIa, thrombin, plasmin, porcine pancreatic elastase, bovine pancreatic chymotrypsin, or bradykinin. Bovine pancreatic trypsin (2 μM) aggregated PMN, but to a lesser extent than kallikrein (0.18 μM). Kallikrein was a potent aggregant agent for PMN because similar responses were observed with kallikrein (0.5 U/ml or 0.23 μM) and an optimal dose (0.2 μM) of N-formyl-methionyl-leucyl-phenylalanine. In addition, PMN incubation with kallikrein resulted in stimulation of their oxidative metabolism as assessed by an increased oxygen uptake. Neutropenia and leukostasis observed in diseases associated with activation of the contact phase system may be the result of PMN aggregation by plasma kallikrein.

Schapira, M., Despland, E., Scott, C. F., Boxer, L. A., and *Colman, R. W.*

Journal of Clinical Investigation 69:1199-1202, 1982.

Other support: National Institutes of Health, Swiss National Science Foundation and the Riley Memorial Association.

From the Division de Rhumatologie, Hôpital Cantonal Universitaire, Geneva, Switzerland; Temple University School of Medicine, Philadelphia, and the Division of Pediatric Hematology-Oncology, Indiana University School of Medicine, Indianapolis.

HIGH-MOLECULAR WEIGHT KININOGEN: A SECRETED PLATELET PROTEIN

This study presents immunochemical evidence for the presence of high-molecular weight kininogen (HMWK) in platelets and for HMWK's secretion following platelet activation with a divalent cationophore A23187, collagen, and thrombin. In the first place, human platelets were studied immunochemically to determine if they contain HMWK. On crossed immunoelectrophoresis with total kininogen antisera (antisera that recognizes both high- and low-molecular weight kininogen) extracts of platelets contained total kininogen antigen. Platelet total kininogen antigen showed complete antigenic identity with plasma total kininogen and displayed the same electrophoretic migration as plasma total kininogen. Using antisera monospecific to HMWK, a competitive enzyme-linked immunosorbent assay (CELISA) was developed to directly measure platelet HMWK. By CELISA, 27-101 ng of HMWK antigen per 10^8 platelets were quantitated in detergent-soluble lysates of washed human platelets from nine normal donors with a mean level of 60 ng±24/10^8 platelets. Plasma HMWK, either in the platelet suspending medium or on the surface of the platelets, could only account for 5% of antigen measured in the solubilized platelets. On the CELISA, platelet HMWK was immunochemically identical to plasma and purified HMWK. In another aspect of this study it was shown that platelet HMWK was secreted from platelets after exposure to ionophore A23187, collagen, and thrombin. Secreted platelet HMWK did not become a part of the platelet Triton-insoluble cytoskeleton. On cross immunoelectrophoresis, secreted platelet total kininogen antigen had a similar electrophoretic migration to plasma total kininogen. Thus, human platelets contain HMWK that can be secreted from platelets and that may participate in plasma coagulation reactions.

Schmaier, A. H., Zuckerberg, A., Silverman, C., Kuchibhotla, J., Tuszynski, G. P., and *Colman, R. W.*

Journal of Clinical Investigation 71:1477-1489, 1983.

Other support: National Heart, Lung, and Blood Institute; Clinical Investigator's Award, Temple University Biomedical Research; American Heart Association; National Institutes of Health, and SCOR.

From the Department of Medicine, Hematology/Oncology Section, and the Thrombosis Research Center, Temple University Health Sciences Center, Philadelphia.

REGIONAL MYOCARDIAL BLOOD FLOW DURING NICOTINE INFUSION AFTER CHRONIC CORONARY ARTERY OCCLUSION: EFFECT OF β-ADRENERGIC BLOCKADE

This study was carried out to evaluate the effect of intravenous nicotine on transmural myocardial blood flow in dogs with chronic occlusion of the left anterior descending coronary artery (LAD). In addition, the effect of the β-adrenergic blocking agent, propranolol, on these changes in transmural myocardial blood flow during nicotine infusion was evaluated. To summarize this work, in eight dogs a portion of the left ventricular free wall (LVFW) was rendered collateral-dependent (CD) by gradual occlusion of the left anterior descending coronary artery with a surgically implanted Ameriod constrictor. Six to eight weeks later, the dogs were anesthetized and regional myocardial blood flow was measured with 7-10 μm radioactive microspheres during (a) control conditions, (b) nicotine alone (24 μg/kg/min i.v.), and (c) nicotine (24 μg/kg/min i.v.) after β-adrenergic blockade with propranolol. During control conditions,

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Downey, H. F., Crystal, G

Journal of Cardiovascular

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From the Departments of
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From the Department of Ol
Center, New York.

mean transmural flow was similar in CD, border, and normal regions of the LVFW. Nicotine alone increased flow in all regions of the LVFW with normal (+104%) greater than CD (+56%). These changes in flow were accompanied by increases in mean arterial pressure (+34%) and mean aortic flow (+54%). Nicotine after β-adrenergic blockade appreciably raised mean arterial pressure (+83%) and mean left atrial pressure (+307%), but caused no increase in flow to any region of the LVFW. The results indicate (a) that the nicotine-induced increase in flow is blunted in a CD region, and that (b) β-adrenergic blockade unmasks coronary vasoconstrictor mechanisms during nicotine infusion which prevent increases in flow to normal or CD regions despite increased perfusion pressure and augmented myocardial oxygen demands.

Downey, H. F., Crystal, G. J. and Bashour, F. A.

Journal of Cardiovascular Pharmacology 5(4):685-690, 1983.

Other support: Southwestern Medical Foundation and The Cardiology Fund.

From the Departments of Physiology and Internal Medicine, University of Texas Health Sciences Center, and Cardiovascular Research Laboratory at Methodist Hospital, Dallas.

PURIFICATION AND PROPERTIES OF TWO DIFFERENT α1-PROTEASE INHIBITORS FROM MOUSE PLASMA

Mammalian plasmas contain significant amounts of protein protease inhibitors which serve to regulate several types of extracellular proteolytic activity. In man, these inhibitors have been extensively studied and are well characterized. However, this is not the case for other species. In the murine study presented here, two similar but distinct forms of α1-protease inhibitor (α1-PI) have been isolated and purified 120-fold to homogeneity from the plasma of female, white Swiss mice. The two inhibitors can be separated by chromatography on DEAE-cellulose using a shallow NaCl gradient at pH 8.9 for elution. Because of their differing specificities for elastase and trypsin the two inhibitors have been labeled α1-PI(E) and α1-PI(T), respectively. The apparent M_r for both proteins, as estimated by gel exclusion chromatography, is approximately 53,000 daltons. However by polyacrylamide gel electrophoresis in the presence of SDS, α1-PI(T) has an apparent M_r of 65,000 while the apparent M_r of α1-PI(E) is 55,000. These results suggest differences in charge and carbohydrate composition. The two mouse inhibitors also have different *N*-terminal amino acids. Like human α1-PI, the mouse inhibitors form stable complexes with proteases. However, they differed from human α1-PI in that they were not found to neutralize either human thrombin or plasmin. While α1-PI(E) inhibits bovine pancreatic trypsin, chymotrypsin, and porcine pancreatic elastase, α1-PI(T) is an effective inhibitor only of trypsin. Plasma levels of α1-PI(E) increase significantly 24 hrs. after stimulation of the acute phase reaction while those of α1-PI(T) do not. These data, which suggest that α1-PI(E) and α1-PI(T) are products of different genes, may help interpret the results of experiments in which the mouse has been used as an animal model for human lung disease.

Nathoo, S., Rasums, A., Katz, J., Ferguson, W. S., and *Finlay, T. H.*

Archives of Biochemistry and Biophysics 219(2):306-315, 1982.

From the Department of Obstetrics and Gynecology, New York University Medical Center, New York.

FORMATION AND STABILITY OF THE COMPLEX FORMED BETWEEN HUMAN ANTITHROMBIN-III AND THROMBIN

Antithrombin III (AT-III) is a glycoprotein found in mammalian plasma that inhibits thrombin and certain other serine proteases. In an earlier set of experiments, these investigators found that the inhibition of thrombin by antithrombin-III proceeds by formation of a covalent bond between the two molecules, a bond that is resistant to both reduction and to denaturation by sodium dodecyl sulfate (SDS). The paper presented here shows further that the inhibition of thrombin by antithrombin-III involves formation of a 1:1 covalent complex between protease and inhibitor and concomitant cleavage of the antithrombin-III peptide chain after Arg-385. The resultant fragment remains connected to the complex via a disulfide bond. This complex spontaneously breaks down into a fragment of approximately 55,000 daltons and smaller peptides. Breakdown is prevented by the presence of hydroxylamine or diisopropylflurophosphate, or by denaturation with urea. It occurs even if the purified complex is treated with diisopropylflurophosphate prior to purification, and can be greatly accelerated by the presence of small amounts of active thrombin. The initial sites of proteolytic attack on the complex are after Arg-13 of the thrombin A chain and Arg-68 of the thrombin B chain. These data indicate that active thrombin can be released from the antithrombin-thrombin complex and that thrombin becomes more susceptible to proteolytic attack when complexed with antithrombin.

Ferguson, W. S. and *Finlay, T. H.*

Archives of Biochemistry and Biophysics 220(1):301-308, 1983.

Other support: New York Heart Association.

From the Department of Obstetrics and Gynecology, New York University Medical Center, New York.

LOCALIZATION OF THE DISULFIDE BOND IN HUMAN ANTITHROMBIN III REQUIRED FOR HEPARIN-ACCELERATED THROMBIN INACTIVATION

Antithrombin III (AT-III) contains three disulfide bonds and no free sulfhydryl groups. It was previously reported that only one of the AT-III disulfide bonds is reduced in the absence of denaturing agents. In this study, it is demonstrated that heparin accelerates the rate of inhibition of thrombin by antithrombin III. Reduction of one of the three antithrombin disulfide bonds with dithiothreitol under mild conditions abolishes this rate-enhancing effect without affecting the rate of reaction in the absence of heparin. Alkylation of mildly reduced antithrombin III with [^{3}H]iodacetic acid followed by digestion with cyanogen bromide yielded two major labeled peptides. The smaller peptide, containing Cys-422, was identified as extending from Gly-414 to the C-terminus, Lys-424. These data are consistent with the larger labeled peptide being the one extending from Glu-104 to Met-243 and containing Cys-239. Cys-422 has been shown by other investigators to be linked to Cys-239. These data indicate that the sensitive disulfide bond in antithrombin III extends between Cys-239 and Cys-422; the site at which thrombin cleaves the antithrombin III is between these two half-cystines.

Ferguson, W. S. and *Finlay, T. H.*

Archives of Biochemistry and Biophysics 221(1):304-307, 1983.

Other support: New York Heart Association.

From the Department of Obstetrics and Gynecology, New York University Medical Center, New York.

CIGARETTE SMOKING LIPOPROTEINS

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Mulligan, J. J., Cluette, J.

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CIGARETTE SMOKING IMPAIRS HEPATIC UPTAKE OF HIGH DENSITY LIPOPROTEINS

This investigation focused on tobacco smoke-induced alterations in hepatic uptake and metabolism of high density lipoprotein (HDL). Specifically, the effect of chronic inhalation of cigarette smoke on hepatic uptake of HDL in White Carneau pigeons was examined. Four treatment groups included: (1) Shelf control birds fed a chow diet and retained in their cages; (2) Sham pigeons fed a cholesterol-saturated fat diet and exposed to fresh air by a smoking machine; (3) Low nicotine-low carbon-monoxide (LoLo) animals also fed the cholesterol diet and exposed to low concentrations of these cigarette smoke products; and (4) High nicotine-high carbon monoxide (HiHi) birds fed the cholesterol diet and subjected to high concentrations of these components. Livers from both smoke-exposed groups contained significantly more triglyceride than those from Sham animals while livers from HiHi birds alone had elevated concentrations of protein. Liver slices from LoLo and HiHi pigeons incorporated significantly less HDL ^{3}H free and esterified cholesterol and HDL ^{14}C apoprotein from media during *in vitro* incubation than livers from Sham birds. The results from this study are unique in that they provide the first evidence for a mechanism by which cigarette smoking may impair delivery of HDL to hepatic tissue and thus potentially attenuate anti-atherogenic properties of this lipoprotein.

Mulligan, J. J., Cluette, J. E., Kew, R. R., Stack, D. J., and *Hojnacki, J. L.*

Biochemical and Biophysical Research Communications 112(3):843-850, 1983.

Other support: American Heart Association, Greater Boston Massachusetts Division.

From the Biochemistry Program, Department of Biological Sciences, University of Lowell, Lowell, MA.

ANGIOTENSION-CONVERTING ENZYME: I. NEW STRATEGIES FOR ASSAY

Although the disposition of converting enzyme (kininase II) on the luminal surface of pulmonary endothelial cells is well established and it is recognized that there is a net conversion of angiotensin I into angiotensin II as blood passes through the lungs, little is known at this time about modulations of converting enzyme activity that may arise through, *e.g.*, changes in the quality of inhalants, blood flow or blood oxygenation. In the present effort to develop simpler and more precise means of measuring angiotensin-converting enzyme, a series of acylated tripeptides, each bearing a radioisotope in the moiety used for acylation, was synthesized. Results showed that the enzyme, a dipeptidyl carboxypeptidase, is capable of removing C-terminal dipeptides from a variety of oligopeptides. The following compounds were synthesized here: *p*-I-benzoyl-Gly-Gly-Gly (I), *p*-I-benzoyl-Pro-Phe-Arg (II), *p*-I-benzoyl-Gly-His-Leu (III), *p*-I-benzoyl-Phe-Ala-Pro (IV), *p*-I-benzoyl-Phe-His-Leu (V), and *p*-I-benzoyl-Phe-Ser-Pro (VI). Each of the compounds can be labelled by dehalogenation in $^{3}H_2$ gas. These substrates can be used *in vitro* or *in vivo* to measure converting enzyme. The choice of substrate depends on the goals of the experiment: substrate I or III when wide variations in substrate concentrations are needed, but high sensitivity is not; substrate IV when high sensitivity is needed.

Ryan, J. W., Chung, A. and *Ryan, U. S.*

Environmental Health Perspectives 35:165-170, 1980.

Other support: U. S. Public Health Service and the Hartford Foundation.

From Department of Medicine, University of Miami School of Medicine, Miami, FL.

SIMPLE RADIOASSAYS FOR HUMAN PLASMA AND GLANDULAR KALLIKREINS

Five years ago, a program to develop a specific and sensitive assay for the kallikreins was begun. Through this program, Pro-Phe-Arg-[^{3}H]benzylamide and Pro-Phe-Arg-[^{3}H]anilide (and the corresponding [^{14}C]anilide) were synthesized and developed. Both substrates proved to be highly reactive with human glandular kallikreins, but neither proved to be very reactive with human plasma kallikrein. To obtain a radiolabeled substrate for plasma kallikrein, (D)Pro-Phe-Arg-[^{3}H]benzylamide, a compound modeled after the potent plasma kallikrein alkylating agent, (D)Pro-Phe-Arg-chlormethylketone was prepared. This substrate proved to be even less reactive with human plasma kallikrein than was Pro-Phe-Arg-[^{3}H]benzylamide. Surprisingly, though, it proved to be more reactive with human glandular kallikrein than was the (L)Pro-analog. After this, a search was begun for amino acid residues (and related acyl groups) that might be used to replace Pro or (D)Pro in order to enhance the affinity of substrate for plasma kallikrein. Of twelve acyl groups thus surveyed, <Glu(5-keto-L-Pro) proved to be the best. <Glu-Phe-Arg-[^{3}H]benzylamide is highly reactive with plasma kallikrein. These results indicate that subtle changes in the side-chain of the P_3 subsite of tripeptide substrates for the kallikreins can convey profound changes in selectivity and kinetic behavior. In summary, this paper contains the routine assay protocols for human urinary kallikrein (HUK) and human plasma kallikrein (HPK). The data obtained following these protocols indicate the HUK and HPK have somewhat different requirements in terms of the side-chains of their tripeptide-benzylamide substrates, and there are differences in terms of the substrates themselves.

Ryan, J. W. *et al. (Ryan, U. S.)*

Advances in Experimental Medicine and Biology 156A:241-249, 1983.

From Department of Medicine, University of Miami School of Medicine, Miami, FL.

BRADYKININ-INDUCED RELEASE OF PROSTACYCLIN AND THROMBOXANES FROM BOVINE PULMONARY ARTERY ENDOTHELIAL CELLS: STUDIES WITH LOWER HOMOLOGS AND CALCIUM ANTAGONISTS

The purpose of this study was to investigate the ability of bradykinin to release prostaglandin I_2 and thromboxane A_2 from endothelial cells derived from bovine pulmonary arteries. The results presented in this paper show that bovine pulmonary artery endothelial cells, in serum-free culture medium, release small quantities of prostacyclin and thromboxane A_2 (3-10 and 0.1-0.3 ng/ml; measured as immunoreactive 6-ketoprostaglandin $F_1\alpha$ and thromboxane B_2, respectively). The release of these substances is stimulated by up to 20-fold during a 3 min incubation with the vasodilator, bradykinin. Endothelial cells incubated with [^{3}H]arachidonic acid for 24 h and then exposed to bradykinin for 3 min release ^{3}H into the medium, approximately 65% of which co-chromatographs with 6-ketoprostaglandin $F_1\alpha$ and 3% with thromboxane B_2. The effects of bradykinin are dose-related and are often discernible when the hormone is used at concentrations believed to occur physiologically (10 pg/ml). Furthermore, the bradykinin molecule must be intact; none of its lower homologs affects the release

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of prostacyclin, thromboxane A_2, or ^3H unless used at concentrations (1 μM or higher) unlikely to be achieved *in vivo*. The release appears to involve calcium uptake and calmodulin: it is abolished by EGTA and inhibited by the "slow channel" calcium antagonists, verapamil and nifedipine, and by the calmodulin inhibitor, trifluoperazine. These findings suggest that bradykinin exerts some of its hormonal effects by acting on specific receptors possessed by vascular endothelial cells; receptor activation is associated with calcium transport, arachidonate mobilization, and a selective synthesis of prostacyclin, a vasodilator in its own right.

Crutchley, D. J., Ryan, J. W., *Ryan, U. S.*, and Fisher, G. H.

Biochimica et Biophysica Acta 751:99-107, 1983.

Other support: U. S. Public Health Service.

From the Research Division, Miami Heart Institute, Miami Beach, FL and the University of Miami School of Medicine, Miami, FL.

ASSAY OF ANGIOTENSIN CONVERTING ENZYME *IN VIVO*

Angiotensin converting enzyme (ACE) is known to be capable of hydrolyzing acylated tripeptides, and a standard assay system for ACE has been used for several years now. In the work reported here, however, a series of acylated tripeptides was prepared to bear a radioactive label in their organophilic moieties. Thus, [^3H]hippuryl-His-Leu and [^3H]benzoyl-Phe-Ala-Pro were prepared; the latter being an analog of BPP_{5a}, an ACE substrate of exceptional affinity. Recently, the use of these substrates has been recommended for the assay of ACE *in vivo*. The protocols for these assays are presented in this paper. Results show that the hydrolysis of [^3H]benzoyl-Phe-Ala-Pro during a single passage through the lungs is far more extensive than that of [^3H]hippuryl-His-Leu. For many experimental purposes, a control hydrolysis rate of about 60% provides a useful baseline for examining for variables that may affect the rate of hydrolysis. The hydrolysis of benzoyl-Phe-Ala-Pro *in vivo* is not inhibited by an inhibitor of aminopeptidase A, α-L-Glu-CH_2Br, nor by an inhibitor of carboxypeptidase N, 2-mercaptomethyl-3-guanidinoethylthiopropionic acid. The *in vivo* assays described here are by no means convenient for routine clinical use. However, the assays may well be used in phase I clinical trials and in critically ill patients whose blood pressures are difficult to control. On the basis of the work discussed here, it can be said that given the appropriate cannulation, enzyme activity of any vascular bed can be examined, including those used commonly (*e.g.*, hamster cheek pouch) to study the physiology of the microcirculation. Overall, it is believed that this approach to the assay of selected enzymes *in vivo* has wide applicability and may provide means of improving current understanding of the physical regulation of enzyme activities of living animals.

Ryan, J. W., Berryer, P. and Chung, A. (*Ryan, U. S.*)

Advances in Experimental Medicine and Biology 156B:805-812, 1983.

Other support: U. S. Public Health Service.

From Department of Medicine, University of Miami School of Medicine, Miami, FL.

ANALOGS OF BRADYKININ CONTAINING DEHYDROPHENYLALANINE

Analogs of bradykinin (BK) containing α, β-dehydrophenylalanine (ΔPhe) in place of L-phenylalanine in positions 5 or 8, or 5 and 8 were synthesized and tested in this laboratory recently. All three of these analogs proved to be markedly more resistant than BK to degradation during passage through the pulmonary vascular bed. On the basis of results seen here, it was postulated that ΔPhe, especially when substituted in position 5, conferred resistance to degradation by kininase enzymes. In order to pursue this speculation further, ΔPhe^5-BK, ΔPhe^8-BK, and ΔPhe^{5,8}-BK were examined for their apparent affinities for angiotensin converting enzyme (ACE) (also known as kininase II, the major kininase enzyme of the lungs). Results show that, on the basis of relative I_{50} values, BK itself clearly has a greater affinity for ACE than do the ΔPhe-containing analogs of BK (BK>ΔPhe^8-BK > ΔPhe^5-BK>ΔPhe^{5,8}-BK). Comparable examinations were made for the influence of ACE *in vivo*. In conclusion, on the basis of these and other studies, it is evident that the biologic effects of BK are heavily influenced by kininase enzymes, the most important of which is ACE. BK analogs of high apparent potency, *e.g.*, ΔPhe^5-BK, may owe much of their apparent potency to resistance to enzymic degradation as well as to effective hormone-receptor interactions. Finally, it seems more and more apparent that the interactions of BK and other substrates with ACE can be heavily influenced by rather subtle changes in side-chains or amino acid residues distant from the catalytic site of the enzyme-substrate complex.

Fisher, G. H., Ryan, J. W. and Beryer, P. (*Ryan, U. S.*)

Advances in Experimental Medicine and Biology 156A:607-612, 1983.

Other support: U. S. Public Health Service, American Lung Association, and American Heart Association, Florida Affiliate.

From Department of Medicine, University of Miami School of Medicine, Miami, FL.

EFFECTS OF BRADYKININ AND ITS HOMOLOGS ON THE METABOLISM OF ARACHIDONATE BY ENDOTHELIAL CELLS

Bradykinin can stimulate cellular prostaglandin (PG) biosynthesis. Earlier studies have shown that high doses of BK release PGs from isolated perfused lungs and low doses of BK release PGs from cultured endothelial cells. This study was started to find out whether BK has the ability to release prostacyclin (PGI_2) and other metabolites of arachidonic acid (AA) from cultured pulmonary arterial endothelial cells. BK was also compared here with seven of its lower homologs, including two products formed by angiotensin I converting enzyme (ACE): Arg^1-Phe^5 and Arg^1-Pro^7. Results from thin-layer chromatographic analysis showed that most of the AA released by BK was metabolized to PGI_2, with lesser amounts of $PGF_{2\alpha}$ and TXA_2 being formed. Comparatively, BK was by far the most potent of the peptides studied, the most active homolog, Des-Arg^1-BK, possessing less than 1% of the activity of BK. In summary, the results presented in this paper show that low doses of BK can induce a rapid release of PGI_2 from cultured pulmonary arterial endothelial cells, a finding consistent with the hypothesis that some of the effects of BK on vascular tone may be mediated via release of PGI_2. These results reemphasize the importance of BK-metabolizing enzymes such as ACE, since all of the lower homologs tested had less than 1% of the activity of BK.

Crutchley, D. J., Ryan, J. W., *Ryan, U. S.*, Fisher, G. H., and Paul, S. M.

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Advances in Experimental Medicine and Biology 156A:527-532, 1983.

Other support: U. S. Public Health Service.

From the Research Division, Miami Heart Institute, Miami Beach, FL, and the Department of Medicine, University of Miami School of Medicine, Miami, FL.

DISTANT BINDING SITES OF KININASE II

In this attempt to examine the complexities of the binding sites of kininase II, ongoing studies of the structure-activity relationships of BPP_{9a}, a potent inhibitor of kininase II, have been expanded to include all of the possible lower homologs and selected analogs of BPP_{9a}. For this study the lower homologs and analogs of BPP_{9a} were synthesized by the solid-phase technique, C-terminal amides and C-terminal esters were synthesized in various ways, the final peptides were examined for purity, and inhibitory potencies were measured. Results showed, among other things, that five of the lower homologs are as potent as BPP_{9a} itself. The nature of the last three amino acid residues comprising the C-terminus appears to be important for significant interaction of the peptide with the active site of the enzyme. Although it was previously believed that a free C-terminal carboxyl group was necessary for inhibitory activity, it was found here that there are peptides, each with a blocked C-terminus (ester or amide), which retain high kininase II inhibitory activities. Among the many other observations considered in this paper is that tryptophan at position two of BPP_{9a} is critical for binding of the peptide to the enzyme. However, tryptophan can be replaced in BPP_{9a} by other aromatic acids, such as phenylalanine or tyrosine, with retention of inhibitory activity. Other things noted include: (1) the length of the side chain group at residue six does not appear to be critical. (2) Size *per se* of a peptide does not appear to be a critical factor in terms of the affinity of binding. (3) Certain peptide sequences have been found in which a free C-terminal carboxyl group is not necessary for binding; for example, the C-terminal amides or esters of the (1-9), (2-9), (1-7), and (2-7) lower homologs are potent inhibitors. All of these sequences contain tryptophan. These data, therefore, suggest the importance of additional binding site(s) at a distance from the catalytic site of the enzyme, one filled well by an aromatic amino acid such as tryptophan. The requirements for binding to the distant site(s) appear to differ in some respects from those for the proximal sites, and (4) A positively-charged residue at the active site of the enzyme is thought to bind to the negatively-charged C-terminal carboxyl group of BPP_{9a}. Binding of the free α-carboxyl group of the C-terminal residue may be the first step to enzyme-inhibitor interaction but may not be an absolute requirement for binding. Overall, it seems, within limits, that these investigators have begun to strengthen the hypothesis that compounds can be made which occupy the distant binding site(s) but not those at or near the catalytic site. Presumably bradykinin, a nonapeptide, and angiotensin I, a decapeptide, require access to the distant binding site(s) as well as the proximal site.

Fisher, G. W. and Ryan, J. W. (*Ryan, U. S.*)

In: Fritz, H., Back, N., Dietze, G., and Haberland, G. L. (eds.): *Kinins-III Pt. B*, New York: Plenum Publishing Corporation, 1983, pp. 813-821.

Other support: U. S. Public Health Service and American Heart Association, Florida Affiliate.

From the Department of Medicine, University of Miami, Miami, FL.

A PEPTIDE FORMED BY FIBRINOLYSIS INCREASES MICROVASCULAR PERMEABILITY AND INHIBITS ANGIOTENSIN CONVERTING ENZYME

It is shown in this paper that a fibrin(ogen) pentapeptide, Ala-Arg-Pro-Ala-Lys, increases microvascular permeability and is an inhibitor of angiotensin converting enzyme. This peptide and a variety of its synthetic analogs and homologs were assayed for their abilities to inhibit angiotensin converting enzyme *in vitro*, and the resulting data were compared to the abilities of these compounds to increase microvascular permeability. Although the parent peptide and many of its analogs and homologs were moderately potent inhibitors of the enzyme, no clear relation was established between enzyme inhibition and direct effects of the peptides on microvascular permeability.

Saldeen, T., Ryan, J. W. and Ragnarsson, U. (*Ryan, U. S.*)

Advances in Experimental Medicine and Biology 156B:829-834, 1983.

Other support: U. S. Public Health Service and the Swedish Medical Research Council.

From the Institutes of Forensic Medicine and Biochemistry, University of Uppsala, Uppsala, Sweden, and the Department of Medicine, University of Miami School of Medicine, Miami, FL.

ASSAYS OF COMPONENTS OF THE KALLIKREIN-KININ SYSTEM BASED ON FIRST ORDER REACTION KINETICS

It is widely recommended that enzyme activities be measured *in vitro* under conditions approximating zero order reactions although reactions like these are unlikely to occur *in vivo*. Indeed, *in vivo* the reactions between, *e.g.*, bradykinin and angiotensin converting enzyme probably occur in such a way that the substrate concentration, [S], is well-below Km([S]<-Km). Often conditions for true first order reaction kinetics cannot be obtained because of inability to detect and measure substrate utilization and/or product formation. This is especially true for chromogenic substrates capable of yielding a weak chromophore. However, the problem of detecting substrate depletion or product formation is largely overcome when the substrate possesses a radioactive leaving group readily separated from the substrate itself. In the present study, such substrates were used to examine empirically for advantages and disadvantages of measuring enzyme activities *in vitro* under conditions in which So <<Km; conditions similar to those likely to exist *in vivo*. Among the concluding comments of this paper, it is noted that radiolabeled substrates of high specific radioactivity can be used (without carrier) at concentrations well within the range of first order kinetics. The latter use has many advantages, one of which is that radiolabeled substrates, in extremely low concentrations, can be used to measure certain components of the kallikrein-kinin system *in vivo* under conditions that simulate those for, *e.g.*, the formation and inactivation of bradykinin. However, the principles of these assays are general and are by no means limited to the kallikrein-kinin system.

Carlin, G., Ryan, J. W. and Saldeen, T. (*Ryan, U. S.*)

Advances in Experimental Medicine and Biology 156B:797-804, 1983.

Other support: U. S. Public Health Service.

From the Department of Forensic Medicine, University of Uppsala, Uppsala, Sweden, and Department of Medicine, University of Miami School of Medicine, Miami, FL.

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Ryan, J. W., Carlin, G.

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f Medicine, Miami, FL.

POTENTIATION OF BRADYKININ BY INHIBITORS OF ANGIOTENSIN CONVERTING ENZYME

Bradykinin (BK) has a higher affinity for angiotensin converting enzyme (ACE) than does angiotensin I (AI) and, as the better substrate, should compete more effectively with a competitive inhibitor of ACE, and should be more difficult to preserve. However, empirically, inhibitors of ACE are effective as BK potentiating agents at concentrations too low to inhibit discernibly the rate of conversion of AI into angiotensin II (AII). In the present study, the investigators have examined this apparent paradox experimentally and on theoretical grounds. Results of this study have shown that the more efficiently BK is inactivated under control conditions, the more readily its effects can be potentiated by an ACE inhibitor. To be precise, in this study female Sprague-Dawley rats were anesthetized with i.p. pentobarbital and cannulas were placed into a femoral vein for i.v. injections and into a femoral artery for direct recording of blood pressure (BP). Each rat was then tested for its responsiveness (BP effects) to intravenously administered angiotensin II, angiotensin I, and bradykinin. Log dose-response curves were constructed and an angiotensin converting enzyme inhibitor (usually benzoyl-Phe-3-thio-D-isobutyrl-L-Pro, BPTIP) was given intravenously in such a small dose that the BP response to AI would recover to control values over the course of about 45-60 minutes. Following injection of the inhibitor, alternate injections of BK and AI were made. Results showed that as little as 10 ng of BK caused a discernible fall of BP. Although AI did have a measurable effect on BP, overall it is clear that the effects of BK on BP are potentiated well beyond the time of inhibition of ACE as judged by recovery of the BP response to AI.

Ryan, J. W., Carlin, G., Berryer, P., and Chung, A. (*Ryan, U. S.*)

Advances in Experimental Medicine and Biology 156A:613-620, 1983.

Other support: U. S. Public Health Service.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL, and the Department of Forensic Medicine, University of Uppsala, Uppsala, Sweden.

KININS, ENDOTHELIAL CELLS AND CALMODULIN

Since calmodulin, a rather ubiquitous substance, is believed to participate in a vast array of intracellular activities, the present studies were begun to investigate two major questions about this substance: (1) Do bovine pulmonary artery endothelial cells, cells known to be responsive to bradykinin (BK), contain calmodulin? (2) If the cells contain calmodulin, is their responsiveness modulated by pretreatment of the cells with an agent reputed to compete with Ca^{++} for calmodulin binding sites? In the search for these answers, the endothelial cells were examined by immunocytochemical techniques for the subcellular disposition of calmodulin. (Given the large number of fundamental cellular activities believed to be controlled in whole or part by calmodulin, surprisingly little is known of its distribution within cells.) Because endothelial cells grow in monolayer, it was believed that the cells in culture provide an unusually favorable system for localizing calmodulin at the ultrastructural level. Overall, the data presented in this paper show that endothelial cells, like all other eukaryotic cells yet examined, contain calmodulin. The distribution of calmodulin within the cells is

characteristic and well defined. Although these studies have just begun, it is not unreasonable to suggest that endothelial cells in culture may be extraordinarily amenable to efforts to define the functions of calmodulin in terms of cell structure. Firstly, the cells grow in monolayer throughout their life span. Secondly, the cells *in vivo* are exposed to all hormones and any drugs that may be administered systemically to the host organism. Thirdly, the cells are responsive to hormones, *e.g.*, BK, known to affect vascular tone, and at least one of the responses to BK (release of PGI_2) is inhibited by trifluoperazine; and fourthly, the intact cells, as opposed to disrupted tissue preparations, can be used for concurrent biochemical and morphologic studies.

Ryan, U. S. and Ryan, J. W.

In: Fritz, H., Back, N., Dietze, G., and Haberland, G. L. (eds.): *Kinins-III Pt. A*, New York: Plenum Publishing Corporation, 1983, pp. 671-679.

Other support: National Institutes of Health.

From Department of Medicine, University of Miami School of Medicine, Miami, FL.

INHIBITION OF THE FORMATION OF ANGIOTENSIN III

In this attempt to improve understanding of the immediate metabolic fate of angiotensin II (and the means by which angiotensin II may be converted into des-Asp^1-angiotensin II; also known as angiotensin III), a series of inhibitors and a radiolabeled substrate for aminopeptidase A have been prepared. Angiotensin II, given intravenously, is known to be more potent as a blood pressure raising agent than is angiotensin III, and it has been found that the inhibitors, a-L-aspartic acid chloromethylketone and a-L-glutamic acid bromomethylketone, can potentiate the blood pressure effects of angiotensin II. Conversely, angiotensin III has been reported by others to be more potent than angiotensin II as an aldosterone secretogogue *in vitro*. However, it seems at this point that it should be feasible to use the inhibitors prepared here to distinguish the aldosterone secretogogue effects of angiotensin II and angiotensin III *in vivo*. In this study, the effects of a-L-Asp-CH_2Cl on the aminopeptidase A and on arginine aminopeptidase activities of rat lung homogenates were noted. As shown here, the arginine aminopeptidase, unlike aminopeptidase A, does not require added Ca^{++}. Results of a companion study show the effects of a-L-Asp-CH_2Br, given in a bolus injection of 2 mg in 50 μL of saline, on the intrinsic activity of plasma aminopeptidase A. These results, expressed in terms of first order enzyme units, provide evidence to indicate that the drug, given i.v., effectively inhibits aminopeptidase A *in vivo*. Also, the effects of a-L-Glu-CH_2Br, given by i.v. infusion, on the blood pressure response to angiotensin II are shown. The results presented here show that apparently specific inhibitors of aminopeptidase A are capable of potentiating the effects of angiotensin II given intravenously. For future research purposes, it should be noted from this work that aminopeptidase A inhibitors, unlike angiotensin converting enzyme inhibitors, affect the renin-angiotensin system but not the kallikrein-kinin system.

Chung, A., Ryan, J. W. and Berryer, P. (*Ryan, U. S.*)

Advances in Experimental Medicine and Biology 156A:693-701, 1983.

Other support: U. S. Public Health Service.

From Department of Medicine, University of Miami School of Medicine, Miami, FL.

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METABOLISM OF BRADYKININ BY ENDOTHELIAL CELLS IN CULTURE

Since inhibitors of angiotensin converting enzyme (ACE) are coming into common clinical use, it is becoming increasingly important to define the metabolic fate(s) of bradykinin (BK) under conditions in which ACE is completely or largely inhibited. Thus, the present study was begun with two goals in mind: (1) to confirm that endothelial cells possess on their surfaces a multiplicity of enzymes capable of inactivating BK and (2) to examine which of these enzymes are functionally significant "kininase" enzymes and remain active in the presence of an ACE inhibitor. The first thing studied for this was the metabolism of [^{3}H]Phe^8-BK by isolated, perfused rat lungs. After suitable preparation, radioactive fractions were collected and then examined by paper electrophoresis at pH 2 and pH 5 and by thin layer chromatography. Authentic standard lower homologs of BK were included in the analysis for comparative purposes. These isolated lung perfusion studies were performed in order to provide a standard of comparison with the metabolism of [^{3}H]Phe^8-BK by endothelial cells in culture. In the second series of experiments, post-confluent cultures of bovine pulmonary endothelial cells were used. Within the limits of these studies, it appears that bovine pulmonary artery endothelial cells in culture metabolize BK to yield products like those formed by isolated perfused rat lungs. In a third series of experiments, the metabolism of [^{3}H]Pro^7-BK by endothelial cells in culture was examined. As expected, the major radioactive metabolite (82% of total radioactivity in medium after 120 min of incubation) was Ser-Pro, the dipeptide formed by ACE from Arg^1–Pro^7, a product of the reaction of ACE with BK. As implied by the results of the above-described experiments, isolated rat lungs and bovine pulmonary endothelial cells do not degrade BK through the action of a carboxypeptidase B-like enzyme. At present, it remains unclear which of the endothelial cell enzymes, other than ACE itself, act as true "kininase" enzymes and which merely act to degrade further lower homologs of BK formed by enzymes of primary importance. Clearly, only those enzymes that degrade BK itself as it occurs physiologically merit the term "kininase."

Ryan, J. W., *Ryan, U. S.*, Chung, A., and Fisher, G. H.

Advances in Experimental Medicine and Biology 156B:775-781, 1983.

Other support: U. S. Public Health Service and the American Lung Association.

From Department of Medicine, University of Miami School of Medicine, Miami, FL.

THIOL-DISULFIDE-DEPENDENT INTERCONVERSION OF ACTIVE AND LATENT FORMS OF RAT HEPATIC 3-HYDROXY-3-METHYLGLUTARYL-COENZYME A REDUCTASE

The significance of thiols in liver cholesterol biosynthesis, particularly in the rate-controlling enzyme hydroxymethylglutaryl-CoA reductase, was investigated here. The studies showed that the hepatic hydroxymethylglutaryl-CoA reductase exists in two forms: active (E_a) and inactive latent (E_i) forms. The interconversion between the two forms of the enzyme is thiol-mediated and the maximal degree of activation of E_i depends on the activating thiol with the order of effectiveness: dithioerythritol = dithiothreitol>glutathione (GSH)>cysteine. E_a is inhibited by oxidized glutathione (GSSG). The degree of the inhibition of E_a by GSSG is proportional to the ratio GSSG/thiol in the reaction. E_i was solubilized from microsomes and purified. Its molecular

weight is estimated to be 104,000 by gel filtration chromatography on Sepharose 6B. The reducing agents $NaBH_4$, dithionite and ascorbate failed to activate E_i. $NaBH_4$ did not inhibit E_a whereas only partial inhibition was caused by ascorbate and dithionite. Soluble E_i binds to both blue dextran/Sepharose 4B and agarose/hexane-3-hydroxy-3-methylglutaryl Coenzyme A affinity resins at low-salt concentrations. By contrast, soluble E_i did not bind to agarose/hexane-hydroxymethylglutaryl-CoA whereas quantitative binding of E_i to blue dextran/Sepharose 4B was still observed at low salt concentrations. These results indicate that thiols are necessary cofactors for hydroxymethylglutaryl-CoA reductase reactions. Their effect on the activation of E_i is not caused by change in the state of aggregation of the enzyme. Rather, the reversible change of the enzyme from E_i to E_a is affected by increasing the affinity of the enzyme to the substrate hydroxymethylglutaryl-CoA.

Dotan, I. and *Shechter, I.*

Biochimica et Biophysica Acta 713:427-434, 1982.

From the Department of Biochemistry, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel.

TISSUE SITES OF DEGRADATION OF APOPROTEIN A-I IN THE RAT

In this paper, apoprotein A-I was labeled with a radiolabeled ligand that remains trapped intracellularly following uptake and degradation of the protein moiety. Specifically, the tissue sites of degradation of apoprotein A-I were determined in the rat *in vivo* using a newly developed tracer of protein catabolism, an adduct of ^{125}I-tyramine and cellobiose, which is then covalently coupled to the protein. This methodology takes advantage of the fact that when a protein labeled with ^{125}I-tyramine-cellobiose is taken up and degraded, the radiolabeled ligand remains trapped intracellularly. Thus radio-iodine accumulation in a tissue acts as a cumulative measure of protein degradation in that tissue. In this instance, apoprotein A-I (apo-A-I) was labeled with tyramine-cellobiose (TC) and the TC-labeled apo-A-I was then reassociated with high density lipoprotein (HDL) *in vivo* by injection into donor animals. After 30 min., serum from donor animals was recovered and injected into recipient rats. TC-labeled apo-A-I in the donor serum was shown to be exclusively associated with HDL. The fractional catabolic rate of ^{125}I-TC-apo-A-I was not significantly different from that of conventionally labeled apo-A-I. The kidney was the major site of degradation, accounting for 39% of the total. The liver was responsible for 26% of apo-A-I catabolism, 96% of which occurred in hepatocytes. The kidney was also the most active organ of catabolism/g of wet weight. The tissues next most active/g of wet weight were ovary and adrenal, a finding that is compatible with a special role of HDL in the rat for delivery of cholesterol for steroidogenesis. Preliminary studies using HDL labeled both with ^{125}I-TC-apo-A-I and [^{3}H]cholesterol ethers again demonstrated high rates of renal uptake of apo-A-I but less than 1% of total ether uptake. It is postulated that the high activity of kidney was not due to uptake of intact HDL particles, but rather to glomerular filtration and tubular reabsorption of free apo-A-I.

Glass, C. K., Pittman, R. C., Keller, A., and *Steinberg. D.*

The Journal of Biological Chemistry 258(11):7161-7167, 1983.

Other support: National Heart, Lung, and Blood Institute.

From the Division of Metabolic Disease, Department of Medicine, University of California, San Diego.

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LIPOPROTEINS AND ATHEROSCLEROSIS: A LOOK BACK AND A LOOK AHEAD

G. Lyman Duff was one of the greats among the pioneers working with the complex problem of atherosclerosis. In this Duff Memorial Lecture, the presenter attempts to approach the atherogenic process through the advances in biochemistry and cell biology which have given researchers the concepts and the techniques with which to frame relevant questions about atherosclerosis. There are three specific hypotheses that are being actively considered as of now–The Lipid Hypothesis, the Endothelial Injury Hypothesis, and the Unified Hypothesis. In defense of the first hypothesis, it is pointed out that lipoproteins, most specifically low density lipoproteins (LDL), can be a sufficient cause of atherosclerosis. This is shown most strongly by the genetic evidence of patients with familial hypercholesterolemia, and the genetic argument has recently been strengthened by the development in Japan of a unique strain of LDL-receptor-deficient rabbits, the Watanabe or WHHL strain. Turning to the other major "school" of atherogenesis, the Endothelial Injury Hypothesis, it is noted that endothelial damage–possibly subtle–causes blood platelets to adhere and aggregate. This causes release of the platelet-derived growth factor (PDGF), discovered by Ross and his coworkers, that stimulates smooth muscle cells to proliferate and to secrete connective tissue matrix elements. Macrophages and endothelial cells have been shown recently to secrete growth factors. Thus, repeated episodes of endothelial damage and repeated bouts of smooth muscle cell replication can lead to the development of the "space-occupying lesion" that is recognized as an atheroma. From the presenter's point of view, these two views of atherogenesis, far from representing two contesting "schools" in the classical sense, are better regarded as simply two faces of a unified Janus hypothesis. There are at least six interactions between these two hypotheses that make them almost inseparable, as can be seen in Figure 4 in this paper. In view of these many potential interactions, some firmly established, others needing additional elaboration, it seems that some version of this middleground Unified Hypothesis should become acceptable to most workers in the atherosclerosis research field. The remainder of this closely considered paper is devoted primarily to LDL and how it may promote atherogenesis.

Steinberg, D.

Arteriosclerosis 3:293-301, 1983.

Other support: National Heart, Lung and Blood Institute.

From the Division of Metabolic Disease, Department of Medicine, University of California, San Diego.

DISSOCIATION OF TISSUE UPTAKE OF CHOLESTEROL ESTER FROM THAT OF APOPROTEIN A-I OF RAT PLASMA HIGH DENSITY LIPOPROTEIN: SELECTIVE DELIVERY OF CHOLESTEROL ESTER TO LIVER, ADRENAL, AND GONAD

Since there has been increasingly impressive evidence that risk of coronary heart disease correlates inversely with plasma high density lipoprotein (HDL) levels, much research has been stimulated on HDL metabolism and the underpinnings of the noted

protective effect. In the work presented here, the metabolic fate of homologous HDL was studied in the rat, tracing the apoprotein A-I (apo A-I) and cholesterol ester moieties simultaneously. The apo A-I was labeled with covalently linked ^{125}I-labeled tyramine cellobiose, which accumulates in the cells degrading the apoprotein; [^{3}H]cholesterol ethers, which cannot be hydrolyzed or mobilized after uptake, were incorporated into the lipid core of reconstituted HDL to reflect the fate of the cholesterol esters. Several lines of evidence, including direct comparison with biologically labeled HDL, are presented to support the validity of this approach. The liver was the major organ of cholesterol ether uptake, accounting for 65% of the total; the adrenal gland and ovary were the most active organs per gram (wet) weight. Uptake of cholesterol ether was 7-fold greater than that of apo A-I in adrenal, 4-fold greater in the ovary, and >2-fold greater in the liver. The remaining tissues took up apo A-I and cholesterol ethers at more nearly equal rates. Transfer of HDL-associated cholesterol ethers and ^{125}I-labeled apo A-I to other lipoprotein fractions was not observed; thus, the results reflect direct uptake from HDL itself. Whereas uptake of low density lipoprotein appears to involve endocytosis of intact particles, uptake of HDL in at least some rat tissues involves additional, more complex, transfer mechanisms.

Glass, C., Pittman, R. C., Weinstein, D. B., and *Steinberg, D.*

Proceedings of the National Academy of Sciences of the United States of America 80:5435-5439, 1983.

Other support: National Heart, Lung, and Blood Institute.

From the Division of Metabolic Disease, Department of Medicine, University of California, San Diego.

ASSOCIATION OF COAGULATION FACTOR V WITH THE PLATELET CYTOSKELETON

In order to investigate a possible interaction of the platelet cytoskeleton with plasma membrane components, these studies focused on two coagulation proteins that have been shown to bind to high affinity receptor sites on the platelet surface: Factor V and Factor Xa. Immunological and functional evidence is presented to show that these receptor sites are also associated with the platelet cytoskeleton. Specifically, triton-insoluble cytoskeletons from thrombin-activated platelets have been shown to contain Factor Va. This conclusion is based on the following evidence (a) a monoclonal antibody to Factor V inhibits the cytoskeleton's ability to potentiate the Factor Xa-catalyzed activation of prothrombin and the ability of cytoskeletons to correct the clotting defect of Factor-V-deficient plasma; (b) the properties of cytoskeletal-associated Factor Va and purified Factor Va are similar; for example, both factors are inhibited by EDTA and heat but not by proteolytic enzyme inhibitors such as DFP; (c) cytoskeletal Factor V is most likely in the activated form, Factor Va, since further treatment of the washed cytoskeletons with thrombin does not increase its Factor V coagulant activity. Furthermore, cytoskeletons bind Factor Xa with a dissociation constant of 10.4×10^{-10} M, a value more consistent for platelet Factor Va than for Factor V. Finally, it is postulated that Factor V arises on the platelet cytoskeleton from a site on the surface of the platelet. This platelet surface is further postulated to bind

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Tuszynski, G. P., W

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Factor Va, Factor Xa, and Ca^{2+} and form the crucial prothrombinase complex that generates thrombin, the penultimate step in fibrin formation.

Tuszynski, G. P., *Walsh, P. N.*, Piperno, J. R., and Koshy, A.

The Journal of Biological Chemistry 257(8): 4557-4563, 1982.

Other support: Department of Health and Human Services.

From the Specialized Center on Thrombosis Research, Temple University School of Medicine, Philadelphia.

FACTOR XI ANTIGEN AND ACTIVITY IN HUMAN PLATELETS

The existence and physiologic relevance of platelet factor XI has been a matter of controversy for about 12 years now. In the work presented here, washed platelets, contaminated with less than 0.20% plasma factor XI, were examined for the presence of factor XI antigen and activity. These platelets contained a factor-XI-like coagulant activity (0.67 ± 0.11 U/10^{11} platelets) that remained constant after successive washings. By means of indirect immunofluorescence, a monospecific antibody to factor XI showed specific staining of both normal platelets and platelets from patients deficient in plasma factor XI. Radiolabeled Triton extracts of washed platelets and labeled purified factor XI solutions were analyzed for factor XI antigen by Staph A immunoprecipitation analysis using antibody to purified factor XI followed by SDS gel electrophoresis. On unreduced gels, the platelet material ran as a single band having an apparent molecular weight of 220,000 daltons, whereas purified plasma factor XI gave a single band at 160,000 daltons. On reduced gels, the platelet material analyzed as a single band at 52,000 daltons, whereas purified factor XI gave a single band of 80,000 daltons. Analysis of a partially purified factor XI preparation from platelets by immunoelectrophoresis revealed that the platelet preparation displayed a slightly lower cathodal electrophoretic mobility at pH 8.6 than did plasma factor XI and yet appeared to possess complete antigenic identity with plasma factor XI. These results indicate that platelets possess a form of factor XI that exists as a disulfide-linked 52,000-dalton tetramer in contrast to the plasma form that circulates as a 80,000-dalton disulfide-linked dimer.

Tuszynski, G. P., Bevacqua, S. J., Schmaier, A. H., Colman, R. W., and *Walsh, P. N.*

Blood 59(6):1148-1156, 1982.

Other support: U. S. Department of Health and Human Services.

From the Specialized Center on Thrombosis Research, Temple University School of Medicine, Philadelphia.

INCREASED CHOLESTEROL BIOSYNTHESIS IN LEUKEMIC CELLS FROM PATIENTS WITH HAIRY CELL LEUKEMIA

Leukemic cells from patients with hairy cell leukemia (HCL), a chronic B-cell leukemia, have an increased cholesterol content when compared with normal human

peripheral blood mononuclear cells (PBMNC). In the studies presented here it is shown that HCL cells synthesize ^{14}C-cholesterol from ^{14}C-acetate precursor in lipoprotein-depleted medium at a rate that is 5-6-fold higher than PBMNC. The increased rate of cholesterol synthesis in HCL cells by comparison with PBMNC persists despite the presence of 100 μg low density lipoprotein (LDL), although cholesterol synthesis in HCL cells and PBMNC is suppressed 60%-70% under these conditions, suggesting that HCL cells possess a normal LDL receptor mechanism. HCL cells mount a subnormal DNA synthetic response to Con A when compared with PBMNC and also show a significantly lower increase in cholesterol biosynthesis in response to Con A exposure. Increased cholesterol synthesis in HCL cells is found in both splenectomized HCL patients and those with intact spleens. It was also shown here that increased cholesterol synthesis in HCL cells cannot be explained by increased loss of newly synthesized cholesterol into the culture medium. In fact, HCL cells retain such cholesterol more avidly than do PBMNC. In a related experiment it was seen that HCL cells and PBMNC are both equally sensitive to cholesterol synthesis inhibition by ML-236B and 25-hydroxycholesterol, but are approximately 10 times more sensitive to ML-236B than are bone marrow MNC. The increased rate of cholesterol synthesis in HCL cells may contribute to their redundant plasma membrane. It seems, therefore, that pharmacologic suppression of cholesterol synthesis in HCL cells could be useful in reversing the abnormal surface properties of these cells.

Yachnin, S. et al.

Blood 61(1):50-60, 1983.

Other support: Nalco Cancer Research Fund, the Leukemia Research Foundation, National Institutes of Health, National Cancer Institute, and the Levin Family Research Fund.

From the Department of Medicine and the Committee on Immunology, University of Chicago School of Medicine, Chicago.

MEVALONIC ACID IN CONJUNCTION WITH HELP FROM NEUTROPHILS INDUCES DNA SYNTHESIS AND CELL CYCLING IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES

Mevalonic acid plays an important role in the regulation of mammalian cell growth and division. Results from earlier studies have even suggested a critical role for mevalonic acid, independent of its conversion to cholesterol, in the regulation of DNA synthesis and cell replication. Evidence presented in this paper shows that mevalonic acid does indeed stimulate DNA synthesis in human peripheral blood lymphocytes which have been isolated by gravity sedimentation of blood and freed of adherent cells by nylon column passage. Human peripheral blood mononuclear cells isolated by the Ficoll-Hypaque technique respond less well, but their response to mevalonic acid can be enhanced by the neutrophil-rich Ficoll-Hypaque-isolated "bottom" cell fraction. The kinetics of mevalonic acid-induced lymphocyte transformation are simlar to those of more classic lymphocyte mitogens. In addition to stimulating lymphocyte DNA synthesis, mevalonic acid produces a population of cells representing all phases of the cell cycle whose morphological characteristics are typical of those seen with more conventional mitogens. The DNA synthetic response of lymphocytes to mevalonic

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acid can be abolished by prior exposure of the lymphocytes to X-irradiation or mitomycin C, while the helper effect of granulocytes is unaffected by either treatment. These observations suggest that mevalonic acid may play a role as a critical substance which supports the propagation of cells programmed to divide, or stimulated to divide by various initiators of cell growth. Also, in susceptible cell populations, mevalonic acid may act as an inducer of the entire program of the cell cycle.

Yachnin, S. and Richman, D. P.

Cellular Immunology 72:248-262, 1982

Other support: U.S. Public Health Service, Muscular Dystrophy Association, and the Nalco Research Foundation.

From the Departments of Medicine and Neurology, Franklin McLean Memorial Research Institute, and the Committee on Immunology, University of Chicago School of Medicine, Chicago.

INHIBITION OF OXYGENATED STEROL ENTRY INTO HUMAN RED CELLS AND LYMPHOCYTES BY ISOLATED SERUM LIPOPROTEINS

The effects of human lipoproteins on the entry of oxygenated sterols into red-cell membranes were studied here using purified lipoprotein fractions. Also, in an attempt to compare this process with that in red cells, the characteristics of oxygenated sterol entry into nucleated human cells and its modulation by serum lipoproteins were studied. Results of these studies show that the uptake of oxygenated sterols by nucleated human cells is comparable in almost every respect to the uptake of oxygenated sterols by human red-cell membranes. The uptake in the two tissues is modulated to a similar extent by such variables as concentration of oxygenated sterol in the incubation medium, temperature, time of exposure, the specific sterol studied, and the lipoprotein composition of the medium in which cellular exposure to oxygenated sterol occurs. In addition, the rank order of efficiency with which various oxygenated sterols enter red cells and lymphocytes is the same, with 7β-hydroxycholesterol entering most efficiently and 25-hydroxycholesterol least efficiently. Both low density lipoproteins (LDL) and high density lipoproteins (HDL), when added to oxygenated sterol-containing medium, effectively diminish the amount of oxygenated sterol taken up by red cells and lymphocytes; LDL is approximately 2.5-4 times more efective than HDL in preventing oxygenated sterol entry into cells. Since oxygenated sterols have been reported to be atherogenic, the modulating effects of lipoproteins on oxygenated sterol uptake by cells, and the alterations in membrane structure and function which ensue, may be a useful model for further study.

Yachin, S., Chung, J. and Scanu, A. M.

Biochimica et Biophysica Acta 713:538-546, 1982.

Other support: U. S. Public Health Service and the Nalco Cancer Research Fund.

From the Departments of Medicine and Biochemistry, and the Committee on Immunology, the University of Chicago, Chicago.

MEVALONIC ACID AS AN INITIATOR OF CELL GROWTH: STUDIES USING HUMAN LYMPHOCYTES AND INHIBITORS OF ENDOGENOUS MEVALONATE BIOSYNTHESIS

Mevalonic acid, which is unique in being the only lymphocyte mitogen known which is also a low molecular weight organic product of normal mammalian cell intermediary metabolism, plays a role in the regulation of mammalian cell replication. In the work reported here, it is shown specifically that mevalonic acid (5×10^{-4}-1×10^{-2} M) stimulates DNA synthesis, morphologic transformation and cell cycling in peripheral blood human lymphocytes. Other organic acid anions which serve as cholesterol and mevalonate precursors are devoid of such effects. Both ML-236B and 25-hydroxycholesterol, inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, inhibit concanavalin A-induced lymphocyte transformation, but only the inhibition of ML-236B can be overcome by exogenous mevalonate. In contrast, only 25-hydroxycholesterol inhibits mevalonate-induced lymphocyte DNA synthesis. The effects of mevalonic acid on lymphocytes cannot be reproduced by isopentenyl adenine or isopentenyl adenosine. Unregulated endogenous cellular synthesis of mevalonic acid may contribute to uncontrolled growth in certain malignant cell lines.

Yachin, S.

Oncodevelopmental Biology and Medicine 3:111-123, 1982.

Other support: The Leukemia Research Foundation, U. S. Department of Energy, U. S. Public Health Service and the Nalco Cancer Research Fund.

From the Department of Medicine, Franklin McLean Memorial Research Institute, and the Committee on Immunology, the University of Chicago, Chicago.

CHOLESTEROL AND MEVALONIC ACID ARE INDEPENDENT REQUIREMENTS FOR THE *IN VITRO* PROLIFERATION OF HUMAN BONE MARROW GRANULOCYTE PROGENITOR CELLS: STUDIES USING ML-236B

ML-236B (compactin) is a competitive inhibitor of 3-hydroxyl-3-methylglutaryl coenzyme A (HMG CoA) reductase, the key regulatory enzyme in the sequence that catalyzes the conversion of acetate to mevalonic acid in cholesterol biosynthesis. This compound causes marked inhibition of human bone marrow granulocyte progenitor cell (CFU-C) proliferation. In the work presented here, the effects of ML-236B on the proliferation of CFU-C *in vitro* were investigated and compared with the effects of 25-hydroxycholesterol, the most potent oxygenated sterol compound studied previously. Also, the action of ML-236B was compared with that of 25-hydroxycholesterol on sterol synthesis in marrow mononuclear cells and the reversibility of the effects of ML-236B by mevalonic acid and cholesterol was examined in an attempt to determine the precise mechanism of inhibition of CFU-C growth by ML-236B. Overall, results from these studies showed that: (1) ML-236B is a potent inhibitor of CFU-C proliferation, DNA synthesis, and cholesterol biosynthesis from acetate precursor in marrow mononuclear cells; (2) the effects of ML-236B are completely reversed by mevalonic acid but not by cholesterol, suggesting that mevalonic acid *per se* or one or more of its nonsterol products is critical for cell growth; (3) the inhibitory effects of 25-hydroxycholesterol on CFU-C proliferation and cholesterol biosynthesis are not solely a result

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Hoffman, P. C., Ri

Blood 61(4):667-67

Other support: Le
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Hoffman, P. C., Richman, C. M., Larson, R. A., and *Yachnin, S.*

Blood 61(4):667-671, 1983.

Other support: Leukemia Research Foundation, the Nalco Cancer Center Research Fund, U. S. Public Health Service, and the National Institutes of Health.

From the Department of Medicine and the Committee on Immunology, University of Chicago, Chicago.

CYTOCHALASIN B IS A POTENT MITOGEN FOR CHRONIC LYMPHOCYTIC LEUKEMIA CELLS IN VITRO

Cytochalasin B, a fungal metabolite, has been shown to be a potent mitogen for malignant lymphocytes from patients with chronic lymphocytic leukemia (CLL). When peripheral blood lymphocytes from 19 patients with CLL of B cell origin (B-CLL) were cultured with 0.5 μg cytochalasin B/ml, significant new DNA synthesis ([^{14}C]thymidine incorporation) occurred in 18. Stimulation indices with cytochalasin B varied widely, but in 11 cases they exceeded those seen with concanavalin A (Con A), phytohemagglutinin, or pokeweed mitogen. In all 11, the mitogenic response to cytochalasin B exceeded that to pokeweed mitogen, which is believed to be a T cell-dependent B cell mitogen. In three cases, the responses to cytochalasin B were 8.6, 3.5, and 2.3 times greater than those to Con A. As with other mitogens, the DNA synthetic response to cytochalasin B was time and dose dependent. Significant mitogenic effects were observed with 0.1-5 μg cytochalasin B/ml with a peak of 0.5-2 μg/ml. Stimulated DNA synthesis was abolished by 1 mM hydroxyurea. Cells from two patients with B-CLL were separated by rosetting with sheep erythrocytes (E). Depletion of E-rosette-positive cells from the CLL cell population abolished the response to Con A but did not affect the response to cytochalasin B. As could be seen here, cytochalasin B is a potent mitogen for B-CLL cells and may be useful in cytogenetic studies of this often indolent neoplasm.

Larson, R. A. and *Yachnin, S.*

Journal of Clinical Investigation 72:1268-1276, 1983.

Other support: U. S. Public Health Service and the Nalco Cancer Research Fund.

From the Department of Medicine and the Committee on Immunology, the University of Chicago, and the Franklin McLean Memorial Research Institute, Chicago.

RATES OF CHOLESTEROL BIOSYNTHESIS ARE RELATED TO EARLY DIFFEREINTIATION IN ACUTE NONLYMPHOCYTIC LEUKEMIA CELLS

The capacity of leukemic cells from 20 patients with acute nonlymphocytic leukemia to synthesize cholesterol from acetate was examined in this study. When the leukemic cell population was classified into subsets (French-American-British [FAB] classification) based on morphology, cytochemistry and cytogenetics, marked differences in the cholesterol biosynthesis rates of each subset were found. As leukemic